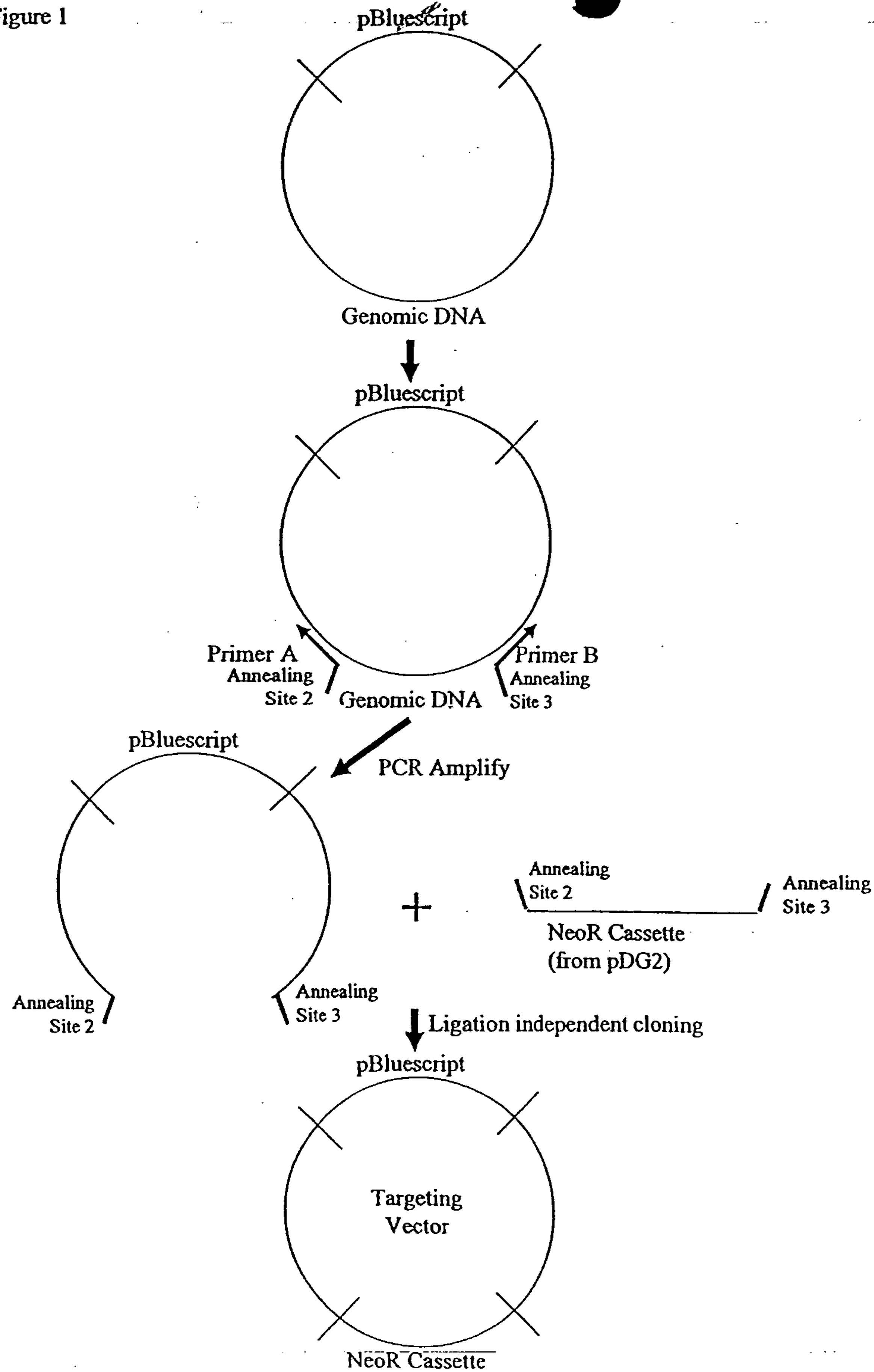
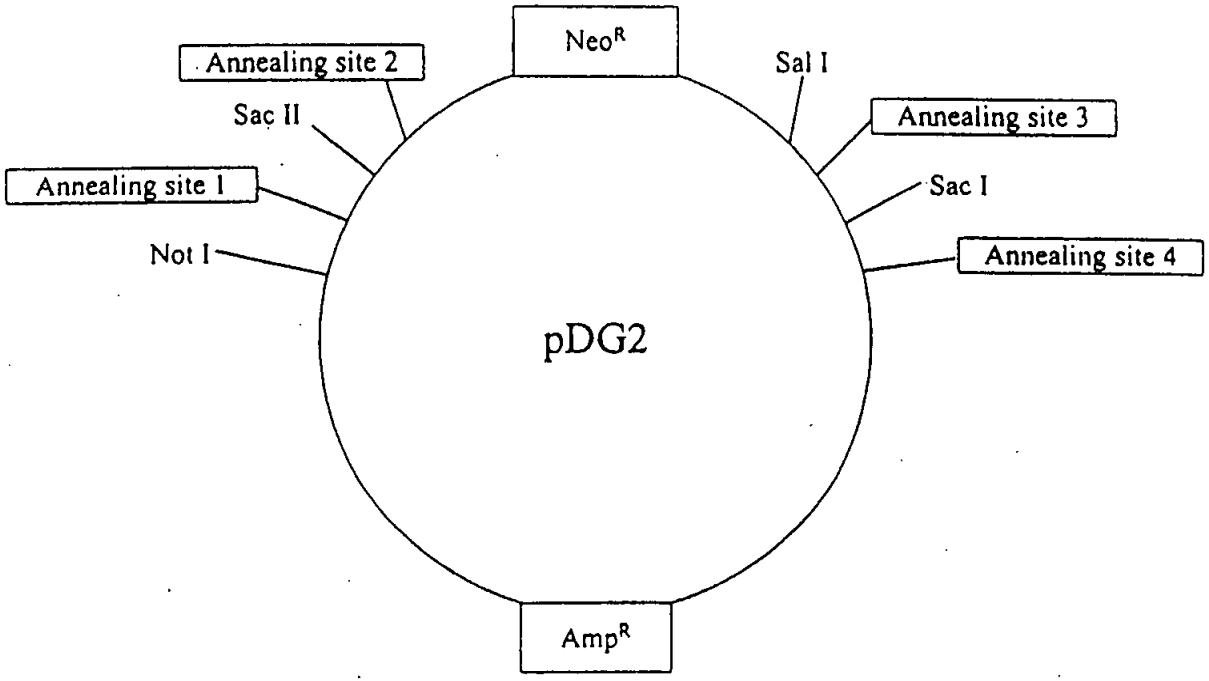
Figure 1


pBluescript
Genomic DNA
pBluescript
Primer A
Annealing
Site 2
Genomic DNA
Primer B
Annealing
Site 3
pBluescript
PCR Amplify
Annealing
Site 2
NeoR Cassette
(from pDG2)
Annealing
Site 3
Annealing
Site 2
Annealing
Site 3
Ligation independent cloning
pBluescript
Targeting
Vector
NeoR Cassette



Plasmid Backbone

FIGURE 2A

FIGURE 2B

pDG2:

GTTAACTACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATA
TGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTC
CGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGA
TGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCC
CCGAAGAACGTTCTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAA
GAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGA
TGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGA
TCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAG
CTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAAC
TGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGC
GCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCA
CTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAG
ACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTG
ATTTACCCCGGTTGATAATCAGAAAAGCCCCAAAAACAGGAAGATTGTATAAGCAAATATTTAAATTGTAAACGTTAATA
TTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTAT
AAATCAAAAGAATAGCCCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTC
CAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCAAATCAAGTTTTTTGGGGT
CGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCGAACGTGGCGA
GAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACA
CCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAA
TCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTT
CTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAAC
TCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACC
ACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAG
TCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCAC
ACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCG
AAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAAC
GCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCG
GAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTAATGTG
AGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATA
ACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTACGTAATACGACTCACTAGGCGGCCGCGTTTAAAC
AATGTGCTCCTCTTTGGCTTGCTTCCGCGGGCCAAGCCAGACAAGAACCAGTTGACGTCAAGCTTCCCGGGACGCGTGCT
AGCGGCGCGCCGAATTCCTGCAGGATTCGAGGGCCCCTGCAGGTCAATTCTACCGGGTAGGGGAGGCGCTTTTCCCAAGG
CAGTCTGGAGCATGCGCTTTAGCAGCCCCGCTGGCACTTGGCGCTACACAAGTGGCCTCTGGCCTCGCACACATTCCACA
TCCACCGGTAGCGCCAACCGGCTCCGTTCTTTGGTGGCCCCTTCGCGCCACCTTCTACTCCTCCCCTAGTCAGGAAGTTC
CCCCCCGCCCCGCAGCTCGCGTCGTGCAGGACGTGACAAATGGAAGTAGCACGTCTCACTAGTCTCGTGCAGATGGACAG
CACCGCTGAGCAATGGAAGCGGGTAGGCCTTTGGGGCAGCGGCCAATAGCAGCTTTGCTCCTTCGCTTTCTGGGCTCAGA
GGCTGGGAAGGGGTGGGTCCGGGGGCGGGCTCAGGGGCGGGCTCAGGGGCGGGGCGGGCGCGAAGGTCCTCCCGAGGCCC
GGCATTCTCGCACGCTTCAAAAGCGCACGTCTGCCGCGCTGTTCTCCTCTTCCTCATCTCCGGGCCTTTCGACCTGCAGC
CAATATGGGATCGGCCATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATG
ACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTC
AAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCC
TTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCC
TGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCT
ACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGA
TGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGATG
ATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGT
GGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATG
GGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGT
TCTTCTGAGGGGATCGATCCGTCCTGTAAGTCTGCAGAAATTGATGATCTATTAAACAATAAAGATGTCCACTAAAATGG
AAGTTTTTCCTGTCATACTTTGTTAAGAAGGGTGAGAACAGAGTACCTACATTTTGAATGGAAGGATTGGAGCTACGGGG
GTGGGGGTGGGGTGGGATTAGATAAATGCCTGCTCTTTACTGAAGGCTCTTTACTATTGCTTTATGATAATGTTTCATAG
TTGGATATCATAATTTAAACAAGCAAAACCAAATTAAGGGCCAGCTCATTCCTCCCACTCATGATCTATAGATCTATAGA
TCTCTCGTGGGATCATTGTTTTTCTCTTGATTCCCACTTTGTGGTTCTAAGTACTGTGGTTTCCAAATGTGTCAGTTTCA
TAGCCTGAAGAACGAGATCAGCAGCCTCTGTTCCACATACACTTCATTCTCAGTATTGTTTTGCCAAGTTCTAATTCCAT
CAGAAGCTGACTCTAGATCTGGATCCGGCCAGCTAGGCCGTCGACCTCGAGTGATCAGGTACCAAGGTCCTCGCTCTGTG
TCCGTTGAGCTCGACGACACAGGACACGCAAATTAATTAAGGCCGGCCCGTACCCTCTAGTCAAGGCCTTAAGTGAGTCG
TATTACGGACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACA
TCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCG
AATGGCGCTTCGCTTGGTAATAAAGCCCGCTTCGGCGGGCTTTTTTTT

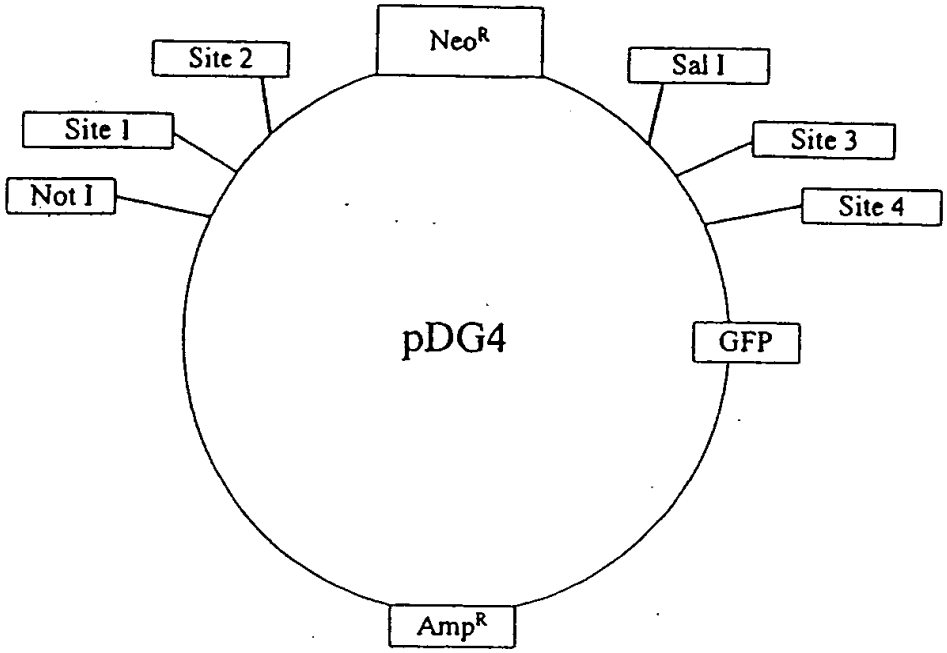


FIGURE 3A

FIGURE 3B

pDG4:

GTTTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGG
CCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGA
CTTTCCAATGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT
ACGCCCCCTATTGACGTCAATGACGGAAAATGGCCCGCCTGGCATTAAGCCCAGTACATGACCTTATGGGACTTTCCTAC
TTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGC
GGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGAC
TTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAG
AGCTGGTTTAGTGAACCGTCAGATCCGCTAGCGCTACCGGTCGCCACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGG
GGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATG
CCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACC
CTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGA
AGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCG
ACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTAC
AACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAA
CATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCG
ACAACCACTACCTGAGGACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTC
GTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTCCGGACTCAGATCCACCGGATCTAGATAACTGAT
CATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATA
AAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTC
ACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTAACGCGAACTACGTCA
GGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCAT
GAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTA
TTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAG
TTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTC
TCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTC
GCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTA
AGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAA
GGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCA
TACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTT
ACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCC
GGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATG
GTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAG
ATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTACCCCGGTT
GATAATCAGAAAAGCCCCAAAAACAGGAAGATTGTATAAGCAAATATTTAAATTGTAAACGTTAATAATTTGTTAAAATT
CGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAAT
AGCCCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGG
CGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCAAATCAAGTTTTTTGGGGTCGAGGTGCCGTAA
AGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCGAACGTGGCGAGAAAGGAAGGGAA
GAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTA
ATGCGCCGCTACAGGGCGCGTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGA
GTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCT
GGTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAG
GTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTC
TGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCG
GGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTG
GAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGC
GGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTT
ATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAA
AACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTAATGTGAGTTAGCTCACTC
ATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACA
GGAAACAGCTATGACCATGATTACGCCAAGCTACGTAATACGACTCACTAGGCGGCCGCGTTTAAACAATGTGCTCCTCT
TTGGCTTGCTTCCGCGGGCCAAGCCAGACAAGAACCAGTTGACGTCAAGCTTCCCGGGACGCGTGCTAGCGGCGCGCCGA
ATTCCTGCAGGATTCGAGGGCCCCTGCAGGTCAATTCTACCGGGTAGGGGAGGCGCTTTTCCCAAGGCAGTCTGGAGCAT
GCGCTTTAGCAGCCCCGCTGGCACTTGGCGCTACACAAGTGGCCTCTGGCCTCGCACACATTCCACATCCACCGGTAGCG
CCAACCGGCTCCGTTCTTTGGTGGCCCCTTCGCGCCACCTTCTACTCCTCCCCTAGTCAGGAAGTTCCCCCCCGCCCCGC
AGCTCGCGTCGTGCAGGACGTGACAAATGGAAGTAGCACGTCTCACTAGTCTCGTGCAGATGGACAGCACCGCTGAGCAA
TGGAAGCGGGTAGGCCTTTGGGGCAGCGGCCAATAGCAGCTTTGCTCCTTCGCTTTCTGGGCTCAGAGGCTGGGAAGGGG
TGGGTCCGGGGGCGGGCTCAGGGGCGGGCTCAGGGGCGGGGCGGGCGCGAAGGTCCTCCCGAGGCCCGGCATTCTCGCAC
GCTTCAAAAGCGCACGTCTGCCGCGCTGTTCTCCTCTTCCTCATCTCCGGGCCTTTCGACCTGCAGCCAATATGGGATCG
GCCATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACA
GACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGT
CCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTG
CTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCT

TGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCG
ACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAA
GAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGATGATCTCGTCGTGAC
CCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTG
TGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTC
CTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGGGGA
TCGATCCGTCCTGTAAGTCTGCAGAAATTGATGATCTATTAAACAATAAAGATGTCCACTAAAATGGAAGTTTTTCCTGT
CATACTTTGTTAAGAAGGGTGAGAACAGAGTACCTACATTTTGAATGGAAGGATTGGAGCTACGGGGGTGGGGGTGGGGT
GGGATTAGATAAATGCCTGCTCTTTACTGAAGGCTCTTTACTATTGCTTTATGATAATGTTTCATAGTTGGATATCATAA
TTTAAACAAGCAAAACCAAATTAAGGGCCAGCTCATTCCTCCCACTCATGATCTATAGATCTATAGATCTCTCGTGGGAT
CATTGTTTTTCTCTTGATTCCCACTTTGTGGTTCTAAGTACTGTGGTTTCCAAATGTGTCAGTTTCATAGCCTGAAGAAC
GAGATCAGCAGCCTCTGTTCCACATACACTTCATTCTCAGTATTGTTTTGCCAAGTTCTAATTCCATCAGAAGCTGACTC
TAGATCTGGATCCGGCCAGCTAGGCCGTCGACCTCGAGTGATCAGGTACCAAGGTCCTCGCTCTGTGTCCGTTGAGCTCG
ACGACACAGGACACGCAAATTAATTAAGGCCGGCCCGTACCCTCTAGTCAAGGCCTTAAGTGAGTCGTATTACGGACTGG
CCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCC
AGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTCGC
TTGGTAATAAAGCCCGCTTCGGCGGGCTTTTTTTT

FIGURE 3B (Continued)

Annealing site	Sequence	Sequence after digestion
1	5' tgtgctcctctttggcttgcttccaa... 3' 3' acacgaggagaaaccgaacgaaggtt... 5'	5' tgtgctcctctttggcttgcttccaa... 3' 3' tt... 5'
2	5' ctggttcttgtctggcttggcccaa... 3' 3' gaccaagaacagaccgaaccgggtt... 5'	5' ctggttcttgtctggcttggcccaa... 3' 3' tt... 5'
3	5' ggtcctcgctctgtgtccgttgaa... 3' 3' ccaggagcgagacacaggcaactt... 5'	5' ggtcctcgctctgtgtccgttgaa... 3' 3' tt... 5'
4	5' tttgcgtgtcctgtgtcgtcgaa... 3' 3' aaacgcacaggacacagcagctt... 5'	5' tttgcgtgtcctgtgtcgtcgaa... 3' 3' tt... 5'

FIGURE 4

Annealing site	Sequence	Sequence after digestion
1	5' AAtgtgctcctctttggcttgcttCCGC 3' 3' Ttacacgaggagaaaccgaacgaagg 5'	5' AA 3' 3' Ttacacgaggagaaaccgaacgaagg 5'
2	5' AActggttcttgtctggcttggCCCGC 3' 3' Ttgaccaagaacagaccgaaccggg 5'	5' AA 3' 3' Ttgaccaagaacagaccgaaccggg 5'
3	5' AAggtcctcgctctgtgtccgttGAGCT 3' 3' Ttccaggagcgagacacaggcaac 5'	5' AA 3' 3' Ttccaggagcgagacacaggcaac 5'
4	5' AAtttgcgtgtcctgtgtcgtcGAGCT 3' 3' Ttaaacgcacaggacacagcagc 5'	5' AA 3' 3' Ttaaacgcacaggacacagcagc 5'

FIGURE 5

FIGURE 6

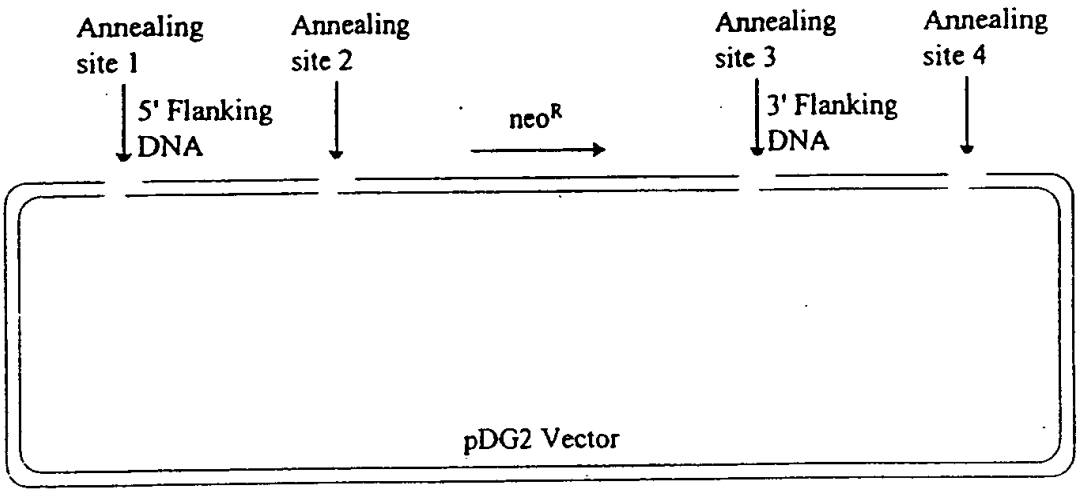
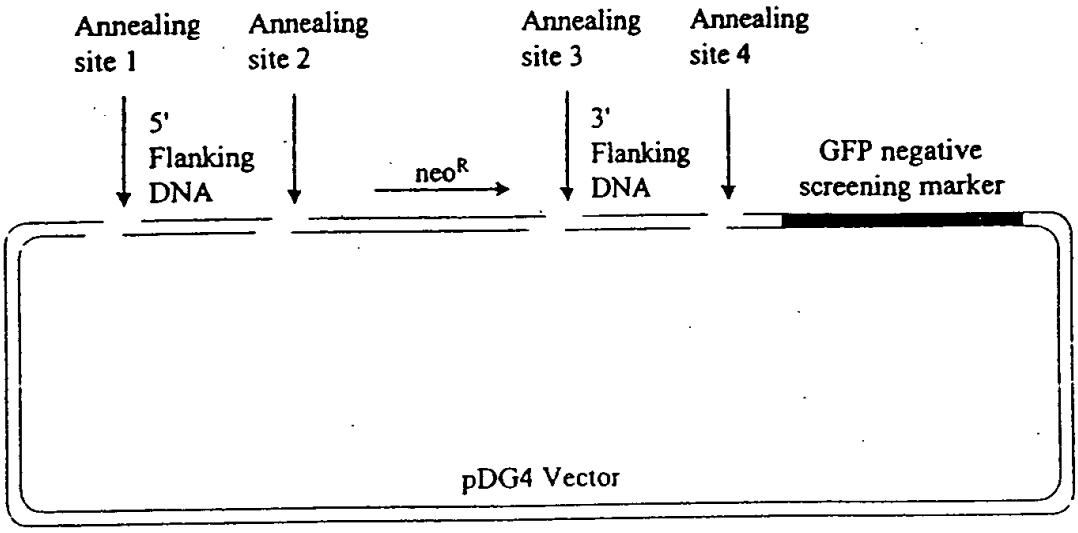


FIGURE 7


Annealing site 1
Annealing site 2
Annealing site 3
Annealing site 4
5' Flanking DNA
neo^R
3' Flanking DNA
GFP negative screening marker
pDG4 Vector

GAATTCCAACCTCAGCTTGACGTGGGGCCTATTGAACTCAATTTGCTTGGAAACTGCCCAGGAAAGGCTG
AGAGCTGAACCCCCTCCTTGGGACAGCTAAAGGGAGTCTTCACCATGGGTGAGGTGACAGCAGAGGAGGT
AGAAAAGTTCCTGGATTCAAATATTGGCTTTGCCAAACAATACTATAACCTTCACTACCGGGGGAAGGTC
ATCTCAGACCTCCTCGGGGCCAAGGAGGCAGCTGTGGACTTCAGCAACTACCACGATGTGAACAGCGTAG
AGGAGAGTGAGATCATCTTTGACCTCCTGCGGGACGTTCAGGAGAACTTACAGGCTGAGAAATGCACATT
CAATGTCATGAAGAAGCTCTGCTTCCTCCTGCGGGCTGACCGAGTGAGCCTGTTCATGTACAGGACCCGC
AACGGCATCGCCGAGCTGGCCACTAGGCTCTTCAATGTCCACAAGGATGCTGTGCTAGAGGACTGCTTGG
TGATGCCCGACTCCGAGATTGTCTTCCCTCTGGACATGGGTGTCGTGGGCCACGTCGCACACTCCAAAAA
GATTGCCAATGTCCCCAACACAGAAGAGGATGAGCATTTCTGTGACTTCGTGGACAATCTCACAGAATAT
CAGACCAAGAACATCCTGGCTTCCCCCATCATGAATGGGAAGGATGTGGTAGCCATAATCATGGCTGTGA
ATAAAATAGATGAACCCCACTTCACCAAGAGAGATGAAGAGATTCTTCTCAAGTACCTCAACTTTGTGAA
CCTGATCATGAAGGTATTCCACCTGAGCTACCTGCACAACTGTGAGACTCGTCGCGGCCAGATATTGCTG
TGGTCTGGGAGCAAGGTCTTTGAGGAGCTCACGGATATAGAGAGGCAGTTCCACAAGGCCCTGTACACGG
TCCGGGCTTTCCTCAACTGTGACAGATACTCCGTAGGACTCTTAGACATGACCAAACAGAAGGAATTTTT
TGATGTGTGGCCAGTTCTGATGGGCGAGGCTCCAGCTTACTCTGGTCCCAGGACTCCAGACGGAAGGGAA
ATTAACTTCTACAAGGTCATTGACTACATCCTGCACGGCAAAGAAGACATCAAAGTCATCCCGAACCCAC
CCGCTGACCACTGGGCTCTAGTGAGTGGTCTACCCCCTTACGTGGCTCAAAATGGTCTGATCTGCAATAT
AATGAATGCGCCTGCAGAGGACTTTTTTGAATTCCAGAAAGAGCCTCTGGATGAGTCTGGGTGGATGATT
AAAAATGTACTCTCCATGCCCATCGTCAACAAGAAGGAAGAGATCGTCGGCGTGGCCACATTTTACAACC
GCAAAGATGGGAAGCCCTTCGACGATATGGACGAGACCCTCATGGAGTCTTTGACTCAGTTTCTGGGATG
GTCAGTCTTAAACCCTGACACCTACGAGTCCATGAACAAGCTCGAGAACAGGAAGGATATCTTCCAGGAC
ATCGTGAAATATCACGTGAAGTGTGATAACGAAGAAATCCAGAAGATCTTGAAAACCAGAGAGGTGTACG
GCAAAGAGCCGTGGGAATGCGAGGAGGAGGAGCTGGCTGAGATCCTGCAAAGAGAACTTCCAGACGCGGA
GTCATACGAAATCAACAAGTTCCACTTCAGCGACCTGCCACTCACGGAGCTGGAGCTGGTGAAGTGCGGC
ATCCAGATGTACTACGAGCTCAGAGTGTGGGACAAGTTCCACATCCCGCAAGAGGCCCTGGTGCGCTTCA
TGTATTCGCTAAGCAAAGGCTACCGGAGAATCACTTACCACAACTGGCGGCATGGCTTCAACGTGGGGCA
GACCATGTTCTCCTTGCTGGTGACAGGAAAGCTGAAACGGTACTTCACTGATCTAGAGGCCTTGGCCATG
GTCACTGCTGCCTTCTGTCATGACATCGACCACAGAGGCACGAACAACCTCTACCAGATGAAATCACAGA
ACCCCCTGGCCAAGCTCCATGGGTCCTCCATCTTGGAAAGGCATCATTTGGAGTTTGGCAAAACACTCCT
GAGAGATGAGAGCCTGAATATCTTCCAGAACCTGAATCGCCGGCAGCATGAGCACGCGATCCACATGATG
GACATCGCGATCATTGCCACAGACCTTGCCTTGTATTTCAAGAAAAGGACCATGTTCCAGAAGATTGTGG
ATCAGTCAAAGACATATGAGAGTACCCAGGAGTGGACCCAGTACATGATGCTGGAGCAGACACGGAAGGA
AATTGTGATGGCCATGATGATGACCGCCTGTGATCTCTCAGCCATCACCAAACCCTGGGAGGTACAGAGC
AAGGTGGCTCTGCTGGTGGCTGCTGAATTCTGGGAGCAAGGTGACCTGGAGCGCACAGTGCTGCAGCAGA
ATCCCATTCCCATGATGGACAGAAACAAGGCGGATGAGCTCCCCAAGCTTCA
AGTCGGCTTCATCGACTTTGTGTGCACTTTTGTCTATAAGGAGTTCTCCCGATTTCATGAGGAGATTACA
CCCATGCTGGATGGGATCACTAACAACCGCAAGGAATGGAAGGCGCTGGCTGATGAGTACGAAGCCAAGA
TGAAGGCCCTGGAGGAGGAGAAGCAGAAGCAGCAGGCAGCCAAGCAAGCTGCTTCCGGGAACCAGCCAGG
AGGGAACCCACTCCAGGGTGCACCTGCATCTAAGTCCTGTTGCATCCAGTAGCTGACTGCACTGCAGCAG
GGCACAGCCCTCAGGAAGGAGGAGGTCACCCTGGCACTGGACAGTTAAAGAACCAGGAGCTTGGAAGTGG
TGGCAAACACAGCAGGCATCTATATCATCAAATGGTCTTAGACATTGGTTCTGTTCTGTTCTGTTCTGTT
CTGTTCTGTTCTGTTCTGTTCTGTTCTGTTCTGTTCTGTTCTGTCCTGTCCTGTTCTGTTCTGTTTTAGA
CAGCTCTGGCTGGCCTGGAACTCTCTATGTAGACTGGGCTGGCCTCAAACTCACAGGCCTCCACCTGCCT
CTGTGTCCTGAGTTCTGAGTTAATAAGCAAGCACCATCACACAGGGACTTAGAGATTGTGTTTAATTCTA
AAAAGTCTATCGAGTCTAGCCTAATATTCTAGACTTCATATACTGACTTGATAATTTTTTGTTCTTATAA
TGCTTGTAATTCTTATAAGCTTTTTAAACTTAGTGTTTTATTATAAAAGTGTTCGCTAATTCCCAAAAGT
ACAGAATTATACGGAATTC (SEQ ID NO:19)

Figure 8A

FIG. 8A

Targeting Vector (5' arm; 200 bp flanking neo insert):

GGAGGTAGAAAAGTTCCTGGATTCAAATATTGGCTTTGCCAAACAGTACTATAACTTTCACTA
CCGGGGGAAGGTCATCTCAGACCTCCTCGGGGCCAAGGAGGCAGCCGTGGACTTCAGCAA
CTACCACGATGTGAACAGCGTAGAGGAGAGTGAGATCATCTTTGACCTCCTGCGGGACGTT
CAGGAGAACTTACAGG (SEQ ID NO:20)

Targeting Vector (3' arm; 200 bp flanking neo insert):

TGTCGTGGGCCACGTCGCACACTCCAAAAAGATTGCCAATGTCCCCAACACAGAAGAGGTACG
CTCTCCCCATAAGATGGATGTACGAATGCACTGTTCCCTGGGGTTCTGGAGTCCAAGCTGGCT
GGGCTGTTGCTGGCCACCAAACCTGGGCTAGTCATAGCACGATACCACTCTCTATTTATAAAAA
ATACTTAGAA (SEQ ID NO: 21)

FIG. 8B